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ABSTRACT

Immunity-related GTPase family M (IRGM) protein, discovered for the first time in 1990, belongs to IRG family of proteins and is divided into five subfamilies (IRGA, IRGB, IRGC, IRGD and IRGM). These are responsive to interferon-γ and play a pivotal role in immune response to pathogens in mice. Owing to lack of interferon response element in the promoter region, human IRGM does not respond to interferon-γ stimulation, which is why it was earlier thought to be non-functional gene. Moreover, evolutionary history suggests that this gene was non-functional for ~25 million years ago. Bioinformatics has been instrumental in elucidating its evolutionary history as well as functions, especially in its interactions with the proteins of autophagy pathway. Recently, several studies have demonstrated the various functions of IRGM in limiting different pathogens in humans. This review discusses how and various roles played by IRGM unraveled in the recent years.

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1. INTRODUCTION

Immunity-related GTPase family M (IRGM) protein belongs to immunity-related GTPase (IRG) family of proteins, which was described first time in 1990s, and is further divided into five subfamilies namely IRGA, IRGB, IRGC, IRGD and IRGM based on homology of GTP-binding domain. Interferons can induce the IRG family genes in mice and these genes have been shown to play a critical role in resistance to a range of intracellular pathogens that comprises *Mycobacterium tuberculosis*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Toxoplasma gondii*, and *Chlamydia trachomatis* (1-4). The IRG proteins consist of an N-terminal terminal GTP-binding domain, called G-domain, and a highly variable C-terminal region. The IRG family G-domain includes all five GTP-binding motifs (5). IRG genes have homology only to conserved G-domain of other GTPases while other regions of these proteins are variable. N- and C-terminal regions of these have characteristic features that distinguish this family of proteins from other P-loop GTPases. The IRG proteins are divided into two structural subfamilies, namely GMS and GKS. The latter is based on a methionine (M) substitution in place of lysine (K) in the G1 motif (GX4GK/MS) and is a typical characteristic of the GMS type protein (6). The IRG family contains 23 genes in mice and these are found as a tandem copies in the genome.

They have been shown to play role in immune response to pathogens and are one of the strongest pathogen resistance systems in mice.

The IRG family in humans contains two genes; an IRGC and truncated gene IRGM. Human IRGM is homologous to mouse IRGM genes of GMS subfamily and is truncated in humans and codes for N- and C-terminally truncated G-domain.

2. HUMAN IRGM GENE STRUCTURAL ORGANIZATION AND ITS EVOLUTION:

The human irgm gene, unlike its mouse ortholog, is not inducible by interferon-gamma (IFN-□) as promoter region has lost the interferons response element (6, 7). IRGM gene is localized at chromosome 5q33.1 and comprises 5 exons, a long coding first exon and 4 shorter exons that extend more than 50 kb downstream from the first exon. This gene could be expressed in five different 3'-spilce isoforms that have different C-terminal ends but their endogenous expression needs to be documented for its all forms. The endogenous IRGM has been shown to localize in mitochondria (8). The mRNA transcripts of five 3'-splicing isoforms (IRGM a - e) extends ~30kb 3' of the first long coding exon. All these different transcripts containing the G-domain of IRGM have been cloned applying 5'- and 3' rapid amplification of cDNA ends (RACE) technology from cultured human cell lines (6, 7). From all known transcripts, IRGMa is the shortest; its termination occurs at the polyadenylation signal sequence at the start of the second intron and made up of 2 exons that encode polypeptide of 181 amino acids. The other transcripts (IRGM b - e) are longer and include two or more downstream exons. Transcripts of this gene have a long 5'- untraslated region (UTR) and consists of promoter region made up by endogenous retroviral nine (ERV9: U3 and U5 region), Alu sequence (AluSc), and a 3′-UTR. The latter encompasses alternatively spliced intronic sequence and exon–intron boundaries and an upstream termination codon in IRGM c – e. Based on the presence of this termination codon, these would be expected to undergo rapid RNA degradation via nonsense-mediated decay. Additionally, there exists a 20.1 kb deletion polymorphism immediately upstream to IRGM gene which has been shown to associate with its altered expression and risk factor for Crohn's disease (9).

Moreover, IRGM has N- and C-terminal regions both with truncation and could not be induced by interferons. This suggested the functions this gene is other than its mouse ortholog. Moreover, because of this reason it was earlier thought be a pseudogene. Bekpen et al (7) have done comprehensive analysis of this gene and have shown that IRG gene cluster was non-functional during primate evolution, approximately 50 million years ago; when the divergence of the anthropoids from the prosimians took place, and the copy number of this gene was reduce to one. Sequence analysis of new and old world monkey species showed that insertion of Alu sequence in the open reading frame (ORF) in the anthropoid common ancestor interrupted this gene making it pseudogene. Further, the function of IRGM was restored, ~20 million years ago, in the ancestors of apes and humans by the insertion of an ERV9 element at 5'- upstream region of IRGM. In addition to this, there are evidences showing the existence of both functional as well as non-functional gene (7, 10). The promoter region corresponds to the ERV9 U3 long terminal repeats which is without interferon response element and could be a possible explanation why this gene is not responsive to the interferons.

3. ROLE OF HUMAN IRGM IN DISEASES:

Earlier, it thought that this gene is non-functional since it was unresponsive to the interferons, unlike other members of its family (6). N- and C-terminal regions of IRGM are truncated which further supports it may have non-functional nature. Moreover, evolutionary history also suggested that this gene was disrupted and non-functional for about 25 million years ago. But, recently scientists have started to unravel the roles played by IRGM and the data suggested critical roles of this gene in controlling infection with the various pathogens in humans (7, 11-14). *Bioinformatics has been instrumental in elucidating its evolutionary history as well as functions especially in its interactions with the proteins of autophagy pathway* (7, 15).

Macroautophagy (referred as autophagy thereafter) pathway which is a conserved eukaryotic catabolic process by which long-lived proteins and damaged organelles are sequestered in the cytoplasm and removed for recycling through lysosomal processes (16). Mechanistically, autophagy starts with the formation of double-membrane vesicles structures called autophagosomes or autophagic vacuoles that engulf cytosolic material, which include damaged organelles or denatured proteins. The autophagosomes so formed fuse with the lysosomes to form single-membrane autolysosomes. Lysosomal enzymes degrade engulfed organelles and regenerate smaller molecules (peptides, amino acids and fatty acids), which are used as a source of energy as well as in anabolic pathways (16-18). Autophagy has been described as a mechanism of both innate and adaptive immunity against intracellular bacteria, viruses, parasites and other diseases (19, 20). Either hosts or

pathogens exploit this process for their benefit and its dysfunction has been implicated in many diseases that include neurodegenerative diseases, muscle diseases, cardiac diseases and infectious diseases (21).

a. IRGM in viral infections:

Currently, sufficient data are available which demonstrate that number of viruses has developed various tactics to modulate autophagy for their benefits. Gre´goire et al (15) has mapped the molecular interactions between the proteins involved in the autophagy pathway and viral proteins. Their data demonstrated the physical interactions between 44 human autophagy–associated proteins and 83 proteins of several RNA viruses belonging to various different families, namely *Bornaviridea, Coronaviridea, Paramyxoviridae, Flaviviridae, Orthomyxoviridae, Retroviridae and Togaviridae*. IRGM was the most commonly targeted protein, interacted with the autophagy pathway proteins, ATG5, ATG10, MAP1CL3C and SH3GLB1 and can interact with 12 viral proteins of Mumps virus (MuV), Hepatits C virus (HCV), Measles virus (MeV), HIV and Chikungunya virus (ChikV) (15). All these proteins regulate one of the earlier steps of the of autophagosome formation, suggesting that IRGM might play a role in the nucleation or the elongation step of autophagic through its interactions with these proteins. These interactions could be facilitated by viral infection (15), which in turn modulate autophagy for their benefit (22).

Infection with MeV induces autophgosomes and MeV virions production could be reduced by inhibiting autophagy suggesting that it uses this pathway to promote its replication (23). siRNA mediated reduction of IRGM expression resulted in decreased levels of MeV replication. Furthermore, it has been shown that IRGM interacts with MeV-C non-structural protein and induces autophagy which implicate MeV might utilize IRGM mediated modulation of autophagy for its replication (15, 23, 24).

HIV-1 also modulates autophagy but the strategy depends on the type of cells infected. It can infect both CD4+ T cells and macrophages resulting in production of high levels of virions. But, its infection leads to death of CD4+ T cells while its cytopathic effects are resisted by macrophages (25, 26). When uninfected CD+4 T cells were co-cultured with lymphocytes expressing Env protein, it induced autophagy that was through binding of Env to its receptors CD4 and CXCR4, but independent of CD4 or CXCR4 signaling pathways that leads to apoptotic cell death of these T cells (27, 28). Additionally, HIV inhibits autophagy in infected CD+ T cells to enhance its replication (28). Dendritic cells (DCs) infected with HIV shows inhibited autophagy and which is through mTOR signaling pathway (29), while in macrophages shows induced autophagy, which is through interactions of HIV-1-NEF and BECN1 (28, 30). Furthermore, NEF proteins can interact with IRGM and overexpression of NEF in HeLa cells shows higher levels of IRGM-dependent autophagosomes accumulation (15). NEF and IRGM has also been shown to co-localize, therefore, NEF-IRGM might be involved in induction of autophagy.

HCV also induces autophagy which is independent of mTOR and inhibition of autophagy results in reduction of autophagy. It has been shown that HCV non-structural proteins NS3-5B induce autophagy. Recent data demonstrated IRGM interactions with HCV-NS3 and siRNA mediated reduction in IRGM expression impair HCV replication (15, 31-36).

Chikungunya virus infection also induces autophagy and is required for its replication, as inhibition and stimulation of the same, respectively, results in impairment and augmentation of the replication of ChikV (37, 38). IRGM interacts with ChikV-NS2 and E3 proteins which point to role of this protein in viral replication that needs to be tested further (15). *Therefore, several viruses target IRGM and manipulate autophagy for their benefit.*

b. Bacterial diseases:

Singh et al (39) for the first time have shown that IRGM is a functional gene and is involved in the control intracellular pathogen, i.e. *Mycobacterium tuberculosis* (*M. tuberculosis*), through autophagy-mediated pathway. They treated U937 cells, human macrophage cell line, with hIFN-γ (which induces autophagy in macrophages) followed by inhibition of IRGM expression using siRNA, a decrease in LC3 puncta were observed compared to controls. They have also shown rapamycin induced autophagy also inhibited LC3II formation suggesting crucial role of IRGM in proper functioning of this pathway (39). Moreover, it has also been shown that IRGM-dependent autophagy induction is needed for *M. tuberculosis* phagosome maturation in human macrophages and knockdown of IRGM results in augmented survival. Therefore, IRGM plays an important role in limiting mycobacteria in macrophages. Further, genome vide association studies have shown the involvement of IRGM polymorphisms in the susceptibility to *M. tuberculosis.* Itemann et al (40) found that *IRGM* -261TT genotype linked to *M. tuberculosis* infection risk but not *M. africanum* clades in a Ghanese

population while Chen et al (19) studied Chinese population and found -1208A/G promoter polymorphisms associated with susceptibility to tuberculosis. King et al (41) showed single nucleotide polymorphism rs10065172 C/T present in IRGM exon region associated with its decreased expression in lymphoblastoid cell line predispose humans to the increased risk to tuberculosis.
Mycobacterium leprae infection of CD4+ T cells, monocytes and monocyte-derived macrophages from leprosy patients were found to have higher levels of both, protein and mRNA of IRGM. Moreover, levels of IRGM were related to the severity of the disease, implicating its role in defending infection of *M. leprae* (12).
Based on data that suggest requirement of autophagy in the clearance of *Salmonella typhimurium* (42, 43), Huett et al., (44) reasoned that IRGM might be involved in controlling infection with this pathogen and have demonstrated that IRGM plays an essential role in autophagy mediated removal of this pathogen. siRNA mediated knockdown of IRGM reduced the number of bacteria within autophagosomes while its overexpression showed increased autophagic activity.

c. Other diseases:

IRGM has also been shown to play a crucial role in Crohn's disease (CD), which is a chronic inflammatory bowel disease. The mechanisms causing this disease are still not completely understood but involve heighten inflammatory responses and impaired bacterial clearance (45, 46). Recent studies have suggested links between autophagy and Crohn's diseases. Genome-wide association studies (GWAS) have revealed sinlge nucleotide polymorphisms (SNPs) in autophagy genes including IRGM, which are known to influence autophagic processing, associated with susceptibility to Crohn's disease (47-49). Several SNPs (rs13361189, rs10065172, rs4958847, rs1000113, rs931058, rs11747270, rs1000113, rs931058 etc.) have been shown to be associated with susceptibility to Crohn's disease in various populations (50-52). Moreover, a protective exonic synonymous SNP (C313T) binds with the miR-196 and downregulates IRGM protective variant but not risk-associated variant. This binding compromises control of replication of intracellular adherent invasive *Escherichia coli* associated with Crohn's disease, predisposing these individuals to higher risk (53).

4. CONCLUSION:

The role of IRGM in humans has started to emerge. Recently, several studies have shown the involvement of this protein in various diseases that includes viral, bacterial, parasitic and inflammatory diseases. *Bioinformatics has played a pivotal role in deciphering its evolution as well as functions of IRGM*. Further detailed studies are needed to find how IRGM is involved in various diseases and to find if this could be targeted to modulated autophagy to control these diseases.

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